Kidney International, Vol. 67 (2005), pp. 631–637

Role of HSD11B2 polymorphisms in essential hypertension and the diuretic response to thiazides

TRACY A. WILLIAMS, PAOLO MULATERO, FABIANA FILIGHEDDU, CHIARA TROFFA, ALBERTO MILAN, GIUSEPPE ARGIOLAS, PAOLO PINNA PARPAGLIA, FRANCO VEGLIO, and NICOLA GLORIOSO

Hypertension Unit, Department of Medicine and Experimental Oncology, University of Torino, Torino, Italy; and Hypertension and Cardiovascular Prevention Center, ASL n. 1, University of Sassari, Sassari, Italy

Role of HSD11B2 polymorphisms in essential hypertension and the diuretic response to thiazides.

Background. The renal 11beta-hydroxysteroid dehydrogenase type 2 (11beta HSD2) enzyme inactivates 11-hydroxy steroids in the kidney, thereby protecting the nonselective mineralocorticoid (MR) receptor from occupation by glucocorticoids. Loss-of-function mutations in the gene encoding 11beta HSD2 (HSD11B2) result in overstimulation of the MR and cause salt-sensitive hypertension.

Methods. We have investigated the role of HSD11B2 in hypertension in 377 genetically homogeneous essential hypertensives from North Sardinia.

Results. Thirty of these patients displayed increased urinary cortisol metabolite ratios (greater than or equal to 2) (tetrahydrocortisol [THF]+allotetrahydrocortisol [aTHF]/tetrahydrocortisone [THE]) reflecting a mild reduction in 11beta HSD2 activity. No mutations in HSD11B2 were detected in these patients. All 377 patients were genotyped for a CA repeat microsatellite in intron 1 of HSD11B2 and a G534A polymorphism in exon 3 of HSD11B2. CA repeat length was associated with the (THF+aTHF)/THE ratio, which in turn was significantly related to PRA levels. No associations were found between the G354A polymorphism and the other parameters. There were no differences in blood pressure (BP) levels between HSD11B2 genotypes, but in a subgroup of 91 patients that underwent diuretic therapy, CA repeat length was strongly associated with the BP response to hydrochlorothiazide.

Conclusion. This study highlights the role of this HSD11B2 polymorphism in sodium handling and is consistent with a role in the BP response to thiazide diuretics.

The nonselective mineralocorticoid receptor (MR) has the same affinity in vitro for its physiologic substrate aldosterone and for the glucocorticoid cortisol [1, 2]. Because tissue concentrations of cortisol are much higher than aldosterone, MR receptor specificity is mediated by the enzyme 11-beta hydroxysteroid dehydrogenase type 2 (11βHSD2), which inactivates cortisol to cortisone [3]. The syndrome of apparent mineralocorticoid excess (AME) is an autosomal-recessive form of salt-sensitive hypertension caused by 11βHSD2 deficiency. In this disorder, cortisol is not inactivated to cortisone and, as a result, binds to the mineralocorticoid receptor in target tissues, such as the distal nephron, thereby causing excessive sodium retention and potassium excretion. The disease is caused by mutations in the 11βHSD2 gene (HSD11B2), most of which markedly decrease or abolish enzyme activity, and lead to an increased urinary excretion of active cortisol (tetrahydrocortisol [THF]) to inactive cortisone (tetrahydrocortisone [THE]) metabolites [4].

Although AME is a rare disorder, it is possible that mutations or polymorphisms with milder effects on 11βHSD2 activity could occur more frequently and be a significant cause of hypertension in the general population. In support of this hypothesis, heterozygous mutations have been described in hypertensive patients in whom the classic features of AME are absent [5], as well as in patients with low renin hypertension [6]. Furthermore, a mild impairment of 11βHSD2 activity has been described in a subgroup of essential hypertensives [7]. Some recent studies have investigated whether alterations in the HSD11B2 gene could play a role in the pathogenesis of essential hypertension. A linkage study using a microsatellite CA repeat marker in intron 1 of the HSD11B2 gene and a silent polymorphism, G534A (Glu178Glu), in exon 3, was performed in French hypertensives, but did not show a significant association of these polymorphisms with hypertension [8]. In contrast, in a study on Italian hypertensives, Agarwal et al [9] found an association of the CA repeat microsatellite allele length with salt-sensitive essential hypertension [9]. In the same study, the authors showed that the ratio of urinary-free cortisol/urinary-free cortisone, used as an index of 11βHSD2 activity, was associated with shorter CA repeat allele length; however, when minigenes,

Key words: 11betaHSD2, cortisol metabolites, pharmacogenomic, genetic polymorphisms.

Received for publication April 27, 2004
and in revised form June 10, 2004, and July 8, 2004
Accepted for publication August 17, 2004

containing either "short" or "long" CA repeats, were transfected into rabbit and human kidney cortical collecting duct cells, the minigenes with short repeats were unexpectedly expressed at higher levels compared to those with long CA repeats [9]. The authors also showed that the urinary-free cortisol/urinary-free cortisone ratio, was lower in 33 salt-sensitive subjects compared to 34 salt-resistant subjects; this association between allele length and salt-sensitivity had been described previously in a different study in young normotensive Germans [10].

To further address the role of the HSD11B2 gene in essential hypertension, we studied 377 genetically homogenous essential hypertensives from North Sardinia. Urinary tetrahydrocortisol (THF), allotetrahydrocortisol (aTHF), tetrahydrocortisone (THE) were measured, and their ratio [(THF+aTHF)/THE] was used as an index of in vivo 11βHSD2 activity. The aims of our study were: (*1*) to investigate if mutations in 11βHSD2 could account for a mild reduction in enzyme activity in essential hypertensive patients; (*2*) to investigate the effects of the CA repeat and G534A polymorphism on blood pressure levels and intermediate phenotypes (plasma renin activity, PRA, (THF+aTHF)/THE); (*3*) to investigate the effects of these polymorphisms on the blood pressure response to thiazide diuretic therapy.

METHODS

Patients

Three hundred seventy-seven never-treated mild-to-severe essential hypertensive patients were enrolled at the Hypertension and Cardiovascular Prevention Center, University of Sassari Medical School, Sassari, Italy, after written informed consent and approval of study protocols by the local Ethics Committee. Patients were Sardinians from at least six generations, born and resident in domains of North Sardinia with a high degree of genetic homogeneity, and were unrelated [11, 12]. In all patients included in the study, the onset of hypertension had occurred before the age of 60, as described previously for another cohort [13]. Essential hypertension was defined as systolic blood pressure (BP) ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg at the end of an eight-week observational period, during which seated BP and heart rate were measured in the morning every two weeks (three measurements per visit, by the same nurse in a quiet room using an automated electronic device). Particular care was taken in the examination of patients' diet to exclude the consumption of food containing glycyrrhetinic acid, such as licorice and grapefruit [14]. In patients with proteinuria in the morning urine sample, proteinuria was quantified in the 24-hour urine collection before inclusion in the study. Patients with more than 30 mg/day of protein leaking were excluded. Secondary hypertension, as well as chronic systemic diseases and clinically relevant acute conditions, were excluded on clinical, laboratory, and instrumental grounds. In particular, cholestasis was excluded by measurement of γglutamyltranspeptidase and sonographic evaluation of the abdomen.

All patients were instructed to keep their sodium intake stable, and underwent a eight-week run-in period; 24-hour urinary sodium was measured at two weekly intervals (accuracy of urine collection was checked by the reproducibility of creatinine clearance), as well as seated blood pressure (BP, the average of the last two readings of four measurements taken at two-minute intervals by the same nurse using an automated electronic device) and body mass index (BMI). "Office" BP at the end of week eight was taken as the reference value. Twenty-four–hour urine and blood samples (at 8 a.m.) were also taken to measure urinary electrolytes and aldosterone, as well as upright PRA (upPRA) and recumbent PRA (recPRA). We also measured plasma cortisol levels in those patients with a (THF+aTHF)/THE ratio >2. A subgroup of 91 patients underwent monotherapy with hydrochlorothiazide (25 mg/day) for eight weeks. The randomization was performed using a table of random numbers without any knowledge of patients' genotype. After four and eight weeks of therapy (weeks 12 and 16), BP levels were compared to baseline levels (after the run-in period, week eight).

Biochemical measurements

All measurements were performed on blood and urine taken at the end of the run-in period (week eight). Urinary THF, aTHF, and THE were measured as described previously [7], and their ratio ((THF+aTHF)/THE) was used as an index of 11βHSD2 activity [15]. The intra-assay and interassay coefficients of variation were 7.8% and 10.3%, respectively. Electrolytes were measured by flame photometry, whereas PRA and plasma aldosterone were measured by radioimmunoassay as described [16, 17]. Plasma cortisol was measured using a RIA kit from Diagnostic Products Corporation (Los Angeles, CA, USA); the normal range at 0800 hours was 10 to 20 μg/dL, with intra- and interassay CVs of 4.1% and 5.0%, respectively.

Genotyping

Blood samples were drawn into EDTA-containing receptacles, and genomic DNA was extracted from peripheral blood leukocytes as described previously [17]. Patients with (THF+aTHF)/THE ratios ≥ 2 ($N = 30$) were identified, and all five exons and intron-exon boundaries of the HSD11B2 gene were amplified by polymerase chain reaction (PCR) and sequenced on both DNA strands as described elsewhere [18]; the promoter region was not sequenced.

The G534A polymorphism located in exon 3 abolishes an *Alu*I restriction site in the presence of the A534 allele [19]. This polymorphism was analyzed in all patients by incubating 10 μL of the PCR product from the amplification of exon 3 with 3U of *Alu*I (New England BioLabs,

Beverly, MA, USA) for 2 hours at 37°C, before analysis of digested products on 3.5% MetaPhor agarose (BioWhittaker Molecular Applications, Rockland, ME, USA) gels.

The CA microsatellite repeat located in intron 1 [20] was analyzed in all patients by PCR amplification of a DNA fragment comprising the CA repeat as described previously [9]. A 6-FAM 5′-labeled sense primer was used for PCR amplifications, and primer sequences, reaction and cycling conditions were as described elsewhere [9]. An aliquot of each PCR product (approximately 10 ng) was mixed with the internal size standard (Applied Biosystems, Foster City, CA, USA) and used for Genescan microsatellite analysis on an ABI prism 3100 DNA sequencer (PE Applied Biosystems). Data were generated using Genotyper software (Applied Biosystems).

Statistical analyses

SAS V8 software (SAS Institute, Inc., Cary, NC, USA) was used for the statistical analyses. Data are expressed as mean ± SD or median (minimum-maximum) when appropriate. The normal distribution of parameters was analyzed by the Kolmogorov-Smirnov test. We used the Fisher exact test to analyze allele frequencies in the various patients' groups.

Differences between two independent variables were evaluated using a *t* test or the Mann-Whitney test when appropriate. Analysis of variance between groups was performed by analysis of variance (ANOVA) among variables, and the Bonferroni test was used to correct for multiple comparison. A nonparametric ANOVA using a Kruskal-Wallis test was used to evaluate differences between variables with nonparametric distribution.

The comparisons between variables were corrected for the common confounding variables (BMI, age, and sex). The Pearson product-moment or a Spearman's correlation coefficient, when appropriate, was used to test the relationship between variables.

RESULTS

Intermediate phenotypes

There was a weak but significant correlation between recPRA and the (THF+aTHF)/THE ratio ($r = -0.09$; $P = 0.04$), whereas there was no statistically significant correlation between upPRA and the (THF+aTHF)/THE ratio ($P = 0.18$). All patients with a (THF+aTHF)/THE ratio ≥2 displayed normal plasma cortisol levels at 8 a.m. (13.2 ± 2.1 μg/dL).

Mutations in HSD11B2 gene

We identified 30 patients with an increased (THF+aTHF)/THE ratio (≥2). To determine if mutations in HSD11B2 could be responsible for the mild reduction of 11βHSD2 activity in these 30 patients, we sequenced the five exons and their intronic flanking regions. Apart from the detection of two polymorphisms (G534A [Glu178Glu] and C468A [Thr156Thr], detected in 3 and 2 patients, respectively, heterozygous in all cases), we did not find any genetic polymorphisms or heterozygous or homozygous mutations in any of the 30 patients analyzed.

Table 1. Clinical and biochemical parameters according to the G534A polymorphism

Genotype	AA+AG	GG	*P* value
N	35	342	
Age *years*	48.3 ± 8.3	47.1 ± 9.7	0.51
BMI kg/m^2	26.3 ± 2.7	27.3 ± 4.1	0.15
SBP *mm Hg*	160.6 ± 13.6	156.9 ± 14.2	0.14
DBP *mm Hg*	107.3 ± 7.3	105.2 ± 8.2	0.14
(THF+aTHF)/THE[a]	1.1 (0.42–2.41)	1.18 (0.27–3.10)	0.38
upALDO *ng/dL*	15.12 ± 6.19	13.39 ± 5.51	0.09
upPRA $ng/mL \times h^{-1}$[a]	1.6 (0.20–5.4)	1.6 (0.2–9.9)	0.59
recPRA $ng/mL \times h^{-1}$	0.93 ± 0.41	0.94 ± 0.59	0.92
UNa+ *mEq/24h*	138.74 ± 51.18	150.92 ± 51.72	0.99
UK+ *mEq/24h*	52.23 ± 15.93	52.23 ± 15.48	0.77

Abbreviations are: BMI, body mass index; SBP, DBP, systolic, diastolic blood pressure before treatment; (THF+aTHF)/THE, (tetrahydrocortisol+allotetrahydrocortisol)/tetrahydrocortisone; upALDO, plasma aldosterone levels in upright position; upPRA, plasma renin activity in upright position; recPRA, plasma renin activity in recumbent position; UNA+, urinary sodium levels; UK+, urinary potassium levels. Values are ± standard deviation.

[a]Data are expressed as median (minimum-maximum value). Differences between groups were evaluated with nonparametric Mann-Whitney test.

G534A polymorphism

The allele frequencies were distributed in agreement with the Hardy-Weinberg equilibrium.

Due to the low frequency of the A allele (only one patient with the AA genotype), the GA and AA genotypes were considered together. We found no difference in blood pressure levels, (THF+aTHF)/THE ratio, and the other parameters between patients with the GG compared to those with the GA+AA genotypes (Table 1). The number of patients in the GA subgroup was too small to be informative for an analysis of the effect of the G534A polymorphism on the diuretic response (9 GA vs. 82 GG).

CA repeat in intron 1

The length of the alleles in our population varied between 12 and 21 CA repeats, with 18 CA repeats being the most frequent (Table 2). Fifty-five genotypes would have been theoretically possible, 28 of which were present in our population. Therefore, we used allele length (defined as "long" or "short") rather than genotype (absolute number of CA repeats) for the analyses in order to increase numbers within the subgroups. Alleles were subdivided into "long" or "short" depending on the length of the CA repeat compared to the median (≤17 or >17 CA repeats), as in Agarwal et al [9]. When patients were subdivided according to the genotype (considered as the allele length: short/short = SS; short/long = SL; long/long = LL), we found a statistically significant difference in the

Table 2. Allele distribution in our population according with the CA repeat length

Allele	*N*	%
12	12	1.59
14	6	0.8
15	24	3.18
16	68	9.02
17	178	23.61
18	385	51.06
19	65	8.62
20	14	1.86
21	2	0.26

(THF+aTHF)/THE ratio between the genotypes ($P = 0.04$), but not for blood pressure levels or for the other biochemical parameters studied (Table 3). Further, if the short allele is considered dominant so that patients are subdivided into two subgroups (SS+SL vs. LL), we found a significantly higher (THF+aTHF)/THE ratio in SS+SL patients compared to patients with the LL genotype ($P = 0.02$) (Fig. 1). The differences remain statistically significant after correction for BMI, age, and sex ($P = 0.01$).

The (THF+aTHF)/THE ratio and the PRA levels in each subject were correlated with the length of the shorter and the longer allele carried by that subject (as if it were a continuous variable); the shorter allele was significantly related to the (THF+aTHF)/THE ratio ($r = -0.14$; $P = 0.006$). We did not find a correlation of the shorter allele with PRA levels, and the longer allele was not related with either the (THF+aTHF)/THE ratio or PRA levels. Further, we only considered alleles carried by more than 30 patients; for the shorter allele, we found a statistically significant trend for a decrease in the (THF+aTHF)/THE ratio with an increasing number of CA repeats ($P = 0.01$). In particular, for allele 16 the ratio was 1.28 [0.56] (median [interquartile difference]), allele 17 = 1.15 [0.58], and allele 18 = 1.11 [0.62]. We also correlated the (THF+aTHF)/THE ratio with the six most common genotypes (i.e., carried by more than 15 patients (16/17, 16/18, 17/17, 17/18, 18/18, 18/19). We found a significant negative correlation between the genotypes and the (THF+aTHF)/THE ratio ($P = 0.02$). Specifically, for the genotype 16/17 the ratio was 1.22 [1.06]; genotype 16/18, ratio = 1.28 [0.43]; 17/17 = 1.18 [0.44]; 17/18 = 1.13 [0.63]; 18/18 = 1.09 [0.7]; 18/19 = 1.15 [0.61].

Response to diuretics

We used two different criteria to group patients arbitrarily into responders (R) and nonresponders (NR) depending on the reduction of the mean blood pressure (MBP) after eight weeks of therapy. The median reduction of the MBP was 14 mm Hg: patients with a reduction of the MBP $\geq$ 14 mm Hg were considered as MBP_{med} R, and patients with a reduction of the MBP <14 mm Hg as MBP_{med} NR. We also considered the median of the percentage reduction of the MBP (MBP_{per} R $\geq$ 10.9%, MBP_{per} NR <10.9%). We used the same criteria for SBP (SBP_{med} R $\geq$ 16 mm Hg, SBP_{med} NR <16 mm Hg; SBP_{per} R $\geq$10.7%, SBP_{per} NR <10.7%) and DBP (DBP_{med} R $\geq$ 12 mm Hg, DBP_{med} NR <12 mm Hg; DBP_{per} R $\geq$ 11.8%, DBP_{per} NR <11.8%).

By analysis of the genotype distribution (when genotypes are considered as the actual number of the CA repeats) between R and NR for SBP, MBP, and DBP, we found a significantly different distribution of the genotypes ($P < 0.01$ for all). The allele distribution (considered as number of CA repeats) between N and NR was significantly different for MBP ($P = 0.038$), but not for SBP and DBP.

RecPRA was significantly correlated to percentage SBP ($r = -0.31$, $P = 0.002$), MBP ($r = -0.28$, $P = 0.007$), but not DBP ($r = -0.22$, $P = 0.06$) reduction, whereas upPRA was only correlated to SBP reduction ($r = -0.20$, $P = 0.04$). The (THF+aTHF)/THE ratio was correlated to percent MBP reduction ($r = 0.2$; $P = 0.049$) and percent DBP reduction ($r = 0.22$; $P = 0.033$) after diuretics but not for the other parameters.

We also analyzed the data considering the BP response after eight weeks of therapy as a continuous variable with alleles dichotomized as "short" or "long" depending on the length of the CA repeat compared to the median repeat length (17 CA repeats), where the short allele is considered as dominant. This analysis demonstrated a significantly higher SBP and MBP reduction under diuretic therapy in patients with the SS+SL genotype ($N = 56$) (-19.3 ± 16 and -15.7 ± 10 mm Hg) compared to those with the LL genotype ($N = 35$) (-15.9 ± 9 and 13.1 ± 7 mm Hg, $P < 0.001$ and $P = 0.01$, respectively) (Fig. 2). Further, patients with the SS+SL genotype displayed a higher percentage SBP and MBP (-11.6 ± 9 and -12.6 ± 8%) reduction under diuretic therapy compared to patients with the LL genotype (-9.9 ± 5 and -10.7 ± 5%, $P < 0.001$ and $P = 0.01$, respectively). There was no difference in the DBP and percentage of the DBP reduction under diuretic therapy between the two groups (SS+SL: -13.8 ± 8 mm Hg and -13.1 ± 8%; LL: -11.8 ± 7 mm Hg and -11.3 ± 6, $P = 0.2$ for both comparisons)(Fig. 2).

We also compared the BP reduction after four weeks of treatment in patients with SS+SL compared to patients with the LL genotype. This analysis demonstrated a significantly higher SBP reduction in patients with the SS+SL genotype (-19.3 ± 17.6 mm Hg) compared to those with the LL genotype (-16.6 ± 11.1, $P < 0.01$). Further, patients with the SS+SL genotype displayed a higher percentage SBP (-11.5 ± 10%) reduction compared to patients with the LL genotype (-10.3 ± 6.5%, $P < 0.01$). There was no difference in the MBP and DBP reduction between the two groups (SS+SL: -16.7 ± 11 and -15.6 ± 15.4 mm Hg; LL: -15.6 ± 8.9 and -14.4 ± 7.6 mm Hg, $P = 0.08$ and 0.3, respectively), despite a trend for a reduction in the MBP.

Table 3. Clinical and biochemical parameters according to the CA repeat allele length

Genotype	SS	SL	LL	*P* value
N (M/F)	51 (30/21)	186 (123/63)	140 (83/57)	
Age *years*	46.8 ± 8.8	47.2 ± 10	47.4 ± 9.2	0.93
BMI kg/m^2	26.6 ± 3.4	27.2 ± 4.1	27.4 ± 3.9	0.45
SBP *mmHg*	154 ± 13	158 ± 15	158 ± 14	0.18
DBP *mmHg*	104 ± 7	106 ± 8	105 ± 8	0.19
THF+aTHF/THE[a]	1.23 (0.5–2.6)	1.19 (0.4–3.1)	1.10 (0.3–2.5)	0.04
upALDO *ng/dL*	12.7 ± 6	14.2 ± 5.4	13 ± 5.6	0.10
recPRA $ng/mL \times h^{-1}$	0.96 ± 0.6	0.91 ± 0.58	0.97 ± 0.57	0.57
upPRA $ng/mL \times h^{-1}$	2.1 ± 1.5	1.9 ± 1.6	2.4 ± 3.7	0.37
UNa+ *mEq/24h*	145.7 ± 43.8	147.4 ± 51.2	154.4 ± 54.9	0.40
UK+ *mEq/24h*	50.7 ± 13.5	52.5 ± 14.9	52.4 ± 16.9	0.76

Abbreviations are: SS, homozygous for short allele (≤17 CA repeats); SL, heterozygous short-long; LL, homozygous for long allele (>17 CA repeats); BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; THF+aTHF/THE, urinary cortisol metabolite ratio (tetrahydrocortisol+allotetrahydrocortisol/tetrahydrocortisone); upALDO, upright aldosterone; upPRA, upright plasma renin activity; recPRA, recumbent plasma renin activity; UNa^+, urinary sodium; UK^+, urinary potassium. Values are ± standard deviation.

[a]Data are expressed as median (minimum-maximum value). Differences between groups were evaluated with nonparametric Kruskal-Wallis test. Differences were also evaluated after logarithmic transformation of the data in order to obtain a normal distribution ($P = 0.03$).

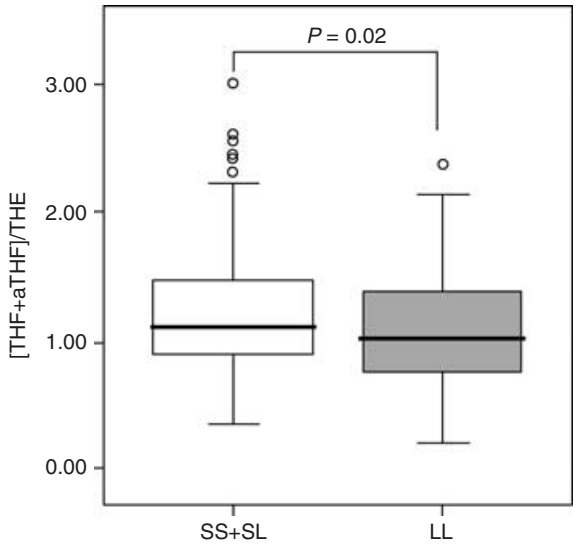

Fig. 1. Effect of CA repeat allele length on urinary cortisol metabolites ratio. Clustering box plot summarizing the differences in (THF+aTHF)/THE levels after subdivision of patients according to genotype. Median value is indicated by the thick line. Box plot and bar indicate 75th and 95th percentile, respectively. SS, homozygous for short allele (≤17 CA repeats); SL, heterozygous short-long; LL, homozygous for long allele (>17 CA repeats). Differences remained significant after correction for BMI, sex, and age ($P = 0.01$), and after log-transformation of the data to obtain a normal distribution ($P = 0.01$).

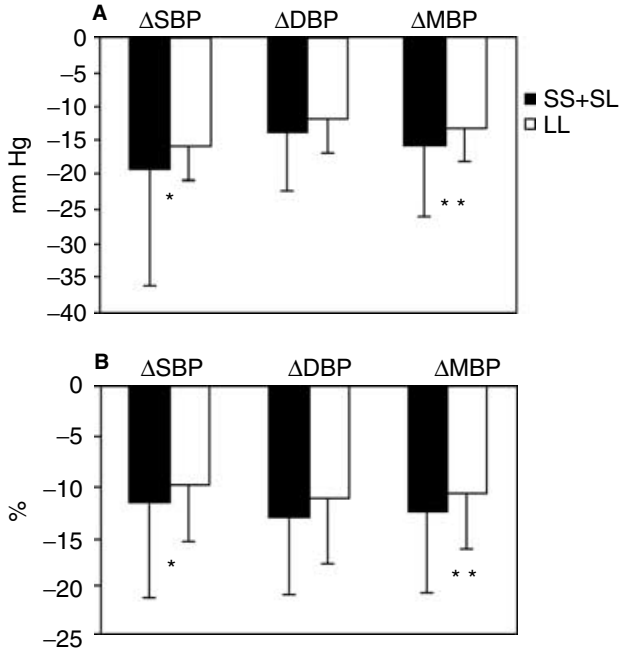

Fig. 2. Effect of CA repeat allele length (*A*) and percentage (*B*) on the response to thiazide diuretic treatment. Differences in absolute blood pressure levels after diuretic treatment and subdivision of patients according to genotype: ■, SS+SL ($N = 56$); □, LL ($N = 35$), where SS is homozygous for short allele (≤17 CA repeats). SL, heterozygous short-long; LL, homozygous for long allele (>17 CA repeats). Bars indicate standard deviation. $^*P < 0.001$; $^{**}P = 0.01$.

DISCUSSION

The present study is to our knowledge the largest study investigating the role of the HSD11B2 gene in essential hypertension. Further, it has been performed in a genetically homogeneous cohort of never-treated individuals, thereby reducing the number of confounders. The geographic location of Sardinia made this island substantially isolated from commercial routes over the past centuries; this led to the development of an isolated population from an evolutionary point of view [21], similar to the Basque and Finnish populations. Our cohort was composed of patients never treated previously for hypertension, thus avoiding effects on phenotypes from the previous use of drugs.

In this large cohort we show an association of a CA repeat polymorphism in the HSD11B2 gene with 11βHSD2 activity and of the (THF+aTHF)/THE ratio with recPRA levels. We also show, in a subgroup of our cohort, an association between this polymorphism and the blood pressure response to thiazide diuretic therapy.

AME is an autosomal-recessive form of salt-sensitive hypertension caused by 11βHSD2 deficiency. The classic form of the disease is very rare; however, mutations

responsible for a mild impairment of 11βHSD2 function could be responsible for the increased blood pressure in some patients with "essential hypertension." This hypothesis is supported by the finding of an increase in cortisol/cortisone metabolites in a subgroup of patients with essential hypertension and by the recent report that patients with a phenotype indistinguishable from essential hypertensives carried mutations in HSD11B2 causing reduced enzyme function [5, 6]. However, we did not find any mutations in the HSD11B2 gene in our hypertensive patients with increased (THF+aTHF)/THE ratios. The slight reduction of 11βHSD2 activity in these patients is therefore more likely due to a difference in HSD11B2 gene expression or in other post-transcriptional regulatory mechanisms, rather than to common mutations; the possibility of an increased cortisol production was ruled out by cortisol measurements. The present data are in agreement with the hypothesis that any differences in total 11βHSD2 activity between hypertensive patients are most probably the result of a reduction in the amount of enzyme with an unaltered activity, rather than by a normal amount of enzyme with a decreased specific activity and confirms the low prevalence of nonconservative mutations found in another European population [22].

The CA repeat polymorphism in intron 1 of the HSD11B2 gene was not associated with BP levels in our genetically homogeneous North Sardinian population. Therefore, in this case at least, it would appear that the HSD11B2 gene does not play a major role in essential hypertension. The CA repeat polymorphism was associated with the (THF+aTHF)/THE ratio, which in turn is significantly related to recPRA values, suggesting a role on sodium balance. This ratio has been demonstrated to be the best indicator of 11βHSD2 activity in vivo, and displays a lower intraindividual variability and a better discrimination between salt-sensitive and salt-resistant subjects compared to the ratio of the urinary free glucocorticoids (UFF/UFE) [15]. The G534A polymorphism was not associated to any of the considered parameters.

The CA repeat polymorphism was strongly associated with the blood pressure response to diuretic therapy with hydrochlorothiazide: patients that carried at least one "short" allele displayed a significantly higher blood pressure reduction after diuretics compared to patients with two "long" alleles. These findings are in agreement with a previous study that found an association of the "short" allele with salt sensitivity [9]. This observation could be accounted for by a genetically determined mild reduction in HSD11B2 gene expression that induces mineralocorticoid receptor activation by cortisol leading to an increase in sodium retention; these patients would be more responsive to diuretic therapy compared to patients in which this pathologic mechanism is not activated.

Among the biochemical variables, recPRA was better than upPRA at predicting the BP response to diuretic therapy and was related to the (THF+aTHF)/THE ratio. The lack of a statistically significant correlation of upPRA with BP response and the (THF+aTHF)/THE ratio is likely due to the effect of posture on the sympathetic system that may randomly affect renin secretion. The association of the PRA status with the BP response to therapy has already been described by others [23]. The (THF+aTHF)/THE ratio showed a weak correlation with the BP response to diuretic therapy; the probable reason for the discrepancy between the effect of the genotype and the effect of the (THF+aTHF)/THE ratio on the BP response to diuretics could be that the ratio is also determined by 11βHSD1 activity.

It may appear surprising that the short allele is dominant because reduced function alleles are usually recessive. However, there are in fact, some examples that are coherent with our findings: (*1*) autosomal-dominant pseudohypoaldosteronism type I is due to a heterozygous loss-of-function mutation in the mineralocorticoid receptor [24]; (*2*) a family with apparent mineralocorticoid excess (AME) has been described where the heterozygous state is associated with an abnormal phenotype (suppressed plasma renin activity and plasma aldosterone level and a moderately elevated urinary cortisol: cortisone metabolite ratio) [25]; (*3*) a patient with mild low-renin hypertension has been described with a homozygous mutation in the HSD11B2 gene. The patient did not demonstrate the typical features of AME and biochemical analyses revealed a moderately elevated cortisol to cortisone metabolite ratio. The conversion of cortisol to cortisone was 58% compared with 0% to 6% in typical patients with AME, whereas the normal conversion is 90% to 95%.

These last two cases indicate that the complete function of both alleles seems to be necessary in order to have a normal conversion of cortisol to cortisone. The model of inheritance of mutations in the HSD11B2 gene may be complex, and other factors can interfere with the phenotypic expression.

A limit of our study is that there is, to date, no demonstration of heritance of the cortisol to cortisone metabolites ratio; however, there is a familial aggregation of low-renin hypertension [26]. For this reason, the association between the HSD11B2 polymorphism and the cortisol to cortisone metabolite ratio and of the ratio with low-renin hypertension can only be inferred indirectly, but is nonetheless plausible.

In transfection experiments using minigenes of HSD11B2 that carried either "short" or "long" alleles, it was the minigenes carrying the short rather than the long allele that resulted in increased levels of 11βHSD2 expression, which appears contradictory to the association of the short CA repeat length with reduced 11βHSD2 activity [9]; however, considering other association studies in humans, the association between short alleles and

salt sensitivity has been confirmed in genetically distinct populations: German and Italian [9], blacks [27], and in Sardinian patients in the present study. A possible explanation for this paradox is that the CA repeat polymorphism in intron 1 is in genetic linkage disequilibrium with another unknown polymorphism or regulatory sequence that has an effect on gene expression that overwhelms any effect of the CA repeat itself [9, 28].

The results of the present study confirm that the CA repeat polymorphism of the HSD11B2 gene might be associated with sodium sensitivity [9, 10], a condition associated with an increased rate of cardiovascular complications [29]. However, our data reinforce and extend this knowledge by showing an association of this genetic polymorphim with the blood pressure response to a thiazide diuretic. Specifically, we show an association of "short" CA repeat alleles with an increased (THF+aTHF)/THE ratio, an index of 11βHSD2 activity, and with a greater blood pressure reduction after diuretic therapy.

CONCLUSION

Thiazide diuretics are still considered the first-line therapy in hypertension treatment; thus, the genetic basis for the response to this type of drug is of particular interest. The results of the present study contribute to an understanding of the complex interaction between genetic polymorphisms and the response to thiazide diuretic treatment.

ACKNOWLEDGMENTS

This work was supported in part by The Italian Society of Hypertension and in part by MIUR (Ministry of University and Research) grant #RBNE01724C_04 FIRB, and by TELETHON grant E.C 471. T.A.W. was in receipt of a grant from the MIUR, Italy. We thank Dr. Aldo Soro for urinary THF, aTHF, and THE measurements.

Reprint requests to Paolo Mulatero, M.D., Hypertension Unit, Department of Medicine and Experimental Oncology, University of Torino, San Vito Hospital, Strada S Vito 34, 10133 Torino, Italy.
E-mail: paolo.mulatero@libero.it

REFERENCES

1. Krozowski ZS, Funder JW: Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Proc Natl Acad Sci U S A* 80:6056–6060, 1983
2. Arriza JL, Weinberger C, Cerelli G, *et al*: Cloning of human mineralocorticoid receptor complementary DNA: Structural and functional kinship with the glucocorticoid receptor. *Science* 237:268–275, 1987
3. Stewart PM, Murry BA, Mason JI: Human kidney 11 beta-hydroxysteroid dehydrogenase is a high affinity nicotinamide adenine dinucleotide-dependent enzyme and differs from the cloned type I isoform. *J Clin Endocrinol Metab* 79:480–484, 1994
4. White PC, Mune T, Agarwal AK: 11 beta-hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev* 18:135–56, 1997
5. Li A, Li KX, Marui S, *et al*: Apparent mineralocorticoid excess in a Brazilian kindred: Hypertension in the heterozygote state. *J Hypertens* 15:1397–1402, 1997
6. Wilson RC, Dave-Sharma S, Wei JQ, *et al*: A genetic defect resulting in mild low-renin hypertension. *Proc Natl Acad Sci U S A* 95:10200–10205, 1998
7. Soro A, Ingram MC, Tonolo G, *et al*: Evidence of coexisting changes in 11 beta-hydroxysteroid dehydrogenase and 5 beta-reductase activity in subjects with untreated essential hypertension. *Hypertension* 25:67–70, 1995
8. Brand E, Kato N, Chatelain N, *et al*: Structural analysis and evaluation of the 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) gene in human essential hypertension. *J Hypertens* 16:1627–1633, 1998
9. Agarwal AK, Giacchetti G, Lavery G, *et al*: CA-repeat polymorphism in intron 1 of HSD11B2: Effects on gene expression and salt sensitivity. *Hypertension* 36:187–194, 2000
10. Lovati E, Ferrari P, Dick B, *et al*: Molecular basis of human salt sensitivity: The role of the 11beta-hydroxysteroid dehydrogenase type 2. *J Clin Endocrinol Metab* 84:3745–3749, 1999
11. Cappello N, Rendine S, Griffo R, *et al*: Genetic analysis of Sardinia: I. Data on 12 polymorphisms in 21 linguistic domains. *Ann Hum Genet* 60:125–141, 1996
12. Piazza A: Who are the Europeans? *Science* 260:9–11,1993
13. Glorioso N, Manunta P, Filigheddu F, *et al*: The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be excluded by a negative association study. *Hypertension* 34:649–654, 1999
14. Lee YS, Lorenzo BJ, Koufis T, Reidenberg MM: Grapefruit juice and its flavonoids inhibit 11 beta-hydroxysteroid dehydrogenase. *Clin Pharmacol Ther* 59:62–71, 1996
15. Ferrari P, Sansonnens A, Dick B, Frey FJ: In vivo 11beta-HSD-2 activity: Variability, salt-sensitivity, and effect of licorice. *Hypertension* 38:1330–1336, 2001
16. Preibisz JJ, Sealey JE, Aceto RM, Laragh JH: Plasma renin activity measurements: An update. *Cardiovasc Rev Rep* 3:787–804, 1982
17. Mulatero P, Schiavone D, Fallo F, *et al*: CYP11B2 gene polymorphisms in idiopathic hyperaldosteronism. *Hypertension* 35:694–698, 2000
18. Mune T, Rogerson FM, Nikkila H, *et al*: Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nat Genet* 10:394–399, 1995
19. Smolenicka Z, Bach E, Schaer A, *et al*: A new polymorphic restriction site in the human 11 beta-hydroxysteroid dehydrogenase type 2 gene. *J Clin Endocrinol Metab* 83:1814–1817, 1998
20. Agarwal AK, Rogerson FM, Mune T, White PC: Gene structure and chromosomal localization of the human HSD11K gene encoding the kidney (type 2) isozyme of 11 beta-hydroxysteroid dehydrogenase. *Genomics* 29:195–199, 1995
21. Cavalli-Sforza LL, Piazza A: Human genomic diversity in Europe: A summary of recent research and prospects for the future. *Eur J Hum Genet* 1:3–18, 1993
22. Zaehner T, Plueshke V, Frey BM, *et al*: Structural analysis of the 11beta-hydroxysteroid dehydrogenase type 2 gene in end-stage renal disease. *Kidney Int* 58:1413–1419, 2000
23. Blumenfeld JD, Laragh JH: Renin system analysis: A rational method for the diagnosis and treatment of the individual patient with hypertension. *Am J Hypertens* 11:894–896, 1998
24. Lifton RP, Gharavi AC, Geller DS: Molecular mechanisms of human hypertension. *Cell* 104:545–556, 2001
25. Li A, Li KX, Marui S, *et al*: Apparent mineralocorticoid excess in a Brazilian kindred: Hypertension in the heterozygote state. *J Hypertens* 15:1397–1402, 1997
26. Fisher ND, Hurwitz S, Jeunemaitre X, *et al*: Familial aggregation of low-renin hypertension. *Hypertension* 39:914–918, 2002
27. White PC, Agarwal AK, Li A, *et al*: Possible association but no linkage of the HSD11B2 gene encoding the kidney isozyme of 11beta-hydroxysteroid dehydrogenase to hypertension in black people. *Clin Endocrinol* 55:249–252, 2001
28. White PC, Agarwal AK, Nunez BS, *et al*: Genotype-phenotype correlations of mutations and polymorphisms in HSD11B2, the gene encoding the kidney isozyme of 11beta-hydroxysteroid dehydrogenase. *Endocr Res* 26:771–780, 2000
29. Morimoto A, Uzu T, Fujii T, *et al*: Sodium sensitivity and cardiovascular events in patients with essential hypertension. *Lancet* 350:1734–1737, 1997